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BIOASSAY OF THE TOXICITY OF INSECTICIDE RESIDUES TO THE CODLING MOTH IN APPLE ORCHARDS

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Tests of the comparative effectiveness of candidate insecticides in controlling the codling moth (Carpocapsa pomonella (L.)) on apple were made by the Yakima, Wash., fruit insect investigation station, in the orchards of local growers. Since the allowable degree of ripe fruit injury was not sufficiently great to show differences between most materials that were statistically significant, methods were developed and standardized for bioassaying in the laboratory the toxicity to codling moth adults, eggs, and newly hatched larvae of residues on leaves, twigs, and immature fruits taken from the orchard plots at intervals after application of the experimental insecticides. The results obtained indicated the toxicity to all stages of the codling moth of candidate insecticides on the different surfaces of trees that the insect normally frequents. The results indicated also the period of time during which the residues killed the insect, and the intervals at which the insecticides should be applied to give economic control of injury. This report describes the bioassay methods used, and the comparative toxicities of candidate materials tested during 1960, 1961, and 1962. This is a report of the results of research work and not a recommendation of any of the materials mentioned.

Hough (1929), Siegler and Munger (1933), and Barnes (1958) reported different methods for assaying differences in toxicity to codling moth larvae of candidate insecticides applied to apple fruits in the laboratory. Lathrop and Sazama (1932) and Steiner (1939) assayed in the laboratory the toxicity to codling moth larvae of insecticide residues on samples of immature fruits taken from orchard plots at intervals after candidate materials had been applied, in addition to determining differences in fruit injury at harvesttime. They gave no attention, however, to the toxicity to adults, eggs, and larvae of residues on leaves and twigs.

General Procedures

The experimental insecticides were applied in sprays to three contiguous rows of trees, about 1 acre in orchard area, with an air blast sprayer that delivered about 44,000 c.f.m. of air from two sides at a speed of

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approximately 100 m.p.h. at the air nozzle. The sprayer was drawn between rows at 1.25 m.p.h.. Sprays were applied at the approximate rate of 800 gallons per acre. A few of the insecticides were applied to 1-tree replicated plots with a hand gun at 500 p.s.i. pump pressure. The insecticides were tested at rates per acre or 100 gallons recommended by State spray bulletins, or by the manufacturer. All of the insecticides were formulated as wettable powders except Shell Development Company SD-5533, which was formulated as an emulsifiable concentrate, and the emulsifiable combination of Volck Supreme oil and ethion. Sprays were applied during the latter part of May, June, and July. The quantities of active material per acre mentioned in table 1 were included in each of the three applications. Guthion was used as the standard each year. Samples of twigs bearing leaves, and samples of fruits were taken at random from the middle row of each plot at tree heights of 6 to 10 feet at weekly intervals after application of sprays, for assay in the laboratory of residue toxicity to codling moth adults, eggs, and newly hatched larvae. All laboratory observations were made in chambers in which the temperature was controlled at 80° F., and the relative humidity at 60 to 70 percent.

The biological assay methods approximated those reported by Brunson, Koblitsky, and Chisholm (1962). Adults and eggs were exposed to the residues on two leaves on each of 10 sample twigs. After all of the leaves except two were removed from the twigs, the distal end of the twig was cut within an inch of the upper leaf, and the basal end cut about 5.5 inches from the petiole of the lower leaf. The basal parts of the sample twigs were bound together with a rubber band in such a manner that the leaves were separated to form an umbrella or canopy effect in order that moths might have easy access to each of the leaves. The cage used was a 16-mesh-wire-screen cylinder, 6 inches in diameter and 9.5 inches long. One end of the cylinder was fitted with a removable screen top. The basal end was fitted into a tin can lid with a flange 1 inch high and with a 1-inch hole in its middle. The tin can lid was permanently attached with solder to a pedestal 5 inches high. The basal end of the bundle of twigs was placed through the hole in the lid into a container of water to maintain freshness of the leaves and twigs, and the hole was made moth-tight with cotton. About 3 inches of twig was exposed within the cage. Fifty moths, consisting of about equal numbers of males and females, were then introduced. All of the test cages were placed in a chamber 6 to 8 feet from a single 7-watt red electric light to induce moth activity and oviposition. Three days after the observations were started, the dead moths were counted and the percentage of mortality compared with the mortality in check cages with nonsprayed leaf twigs to determine the mortality caused by the insecticide residue. Mortality of moths in check cages averaged 7.7 percent.

Each lot of moths, while in the cages, deposited several hundred eggs, mostly on leaves from the experimental plots, thus providing an opportunity to observe the ovicidal effectiveness of the residues. An average of 470 eggs were deposited in the experimental cages and 1,150 in the check cages. After the eggs in the check cages had hatched, the percentages of egg mortality in the experimental and check cages were determined and compared to determine the mortality caused by the insecticide residue. The average mortality of eggs in check cages was 5.6 percent.

The toxicity of residues on leaves and twigs to newly hatched larvae was determined by inducing the larvae to crawl over the leaves and twigs to reach nonsprayed apples, which the survivors entered to feed. All leaves but one were removed from each of six sample twigs. The distal end of the twig was removed immediately above the leaf, and the basal end of each twig cut 7.5 inches from the leaf petiole. The basal end was then pushed through a 1-hole rubber stopper, which was inserted into a bottle of water. At a point 3.5 inches from the leaf petiole, two straight pins (1 inch long) were pushed in opposite directions through the twig on which two nonsprayed, thinning-size apples (about $1\frac{1}{2}$ inches in diameter) were impaled, and held in contact with the twig by rubber bands. The assembly was then suspended on a rack in a vertical position with the leaf end downward. A small book binder clip was attached to the end of the leaf as a weight to hold the leaf in a vertical position, and a piece of wax paper, containing 12 to 14 eggs ready to hatch, was attached to the leaf with a straight pin immediately above the binder clip. The racks bearing several test twigs, or assemblies, were then placed in a chamber in such a position that each assembly was immediately below a 7-watt, white frosted electric light, which was about 14 inches from the eggs attached to the leaves. Each three twig assemblies were enclosed by masonite boards, painted black, to form a chamber 12 x 12 x 18 inches, and open at the top. This chamber was intended to aid in attracting the hatching larvae directly upward to the nonsprayed apples in which the survivors were captured. Four to five days after the observation was started, and when no live larvae could be seen on the experimental and check assemblies, the hatched eggs were counted to determine the number of larvae exposed in each test, and the trap apples were dissected to determine the number of survivors. The results obtained in the experimental and check observations were compared to determine the mortality caused by the insecticide residues. Each test was replicated six times. The egg hatch averaged 94.8 percent, and in the check observations, 74.4 percent of the larvae were found in the trap apples.

The toxicity to larvae of residues on immature apples was determined from 24 samples of fruit taken at random from the plots. Two lots of 12 apples were arranged in the form of a cone on cheese cloth. Small pieces, 5 to 6 in number, of wax paper containing a total of about 50 eggs ready to hatch were inserted between the apples of each subsample in such a position that larvae, upon emerging from the eggs, would have access to each of the apples. The cloth was then drawn tight, tied with a string, and suspended from a rack for 4 to 5 days, at which time the egg hatch was determined, and the apples dissected to count the survivors. The percentages of larvae surviving in the experimental and check (nonsprayed) apples were compared to determine the mortality caused by the insecticide residues. Survival of larvae in the check observations averaged 85.1 percent.

The stages of codling moth used in assaying the toxicity of residues were from a culture of the insect produced continuously in the laboratory on immature apples obtained from commercial orchards during the latter part of June and early July, and stored until needed. Nonsprayed fruits were used in all assay tests to trap surviving larvae, and in all check observations.

Results

The mortality of codling moth adults, eggs, and newly hatched larvae caused by the residue of several experimental insecticides, as determined by assay following the described procedures, is shown in table 1. The results show specific differences between materials in toxicity to different stages of the insect, and the probable period of time over which the different materials might be expected to control fruit injury in orchards. Bioassay in the laboratory proved to be a better index of the potential usefulness of the experimental materials than percentage of fruit injury at harvesttime, which seldom exceeded 8 percent in test plots.

The data in table 1 show the comparative effectiveness of various experimental compounds to the several stages of the codling moth. They show that the stage of codling moth least susceptible to the residues on leaves and twigs was usually the egg, and that adults were less susceptible than newly hatched larvae. Larvae were more susceptible to the residues on fruit than to residues on leaves and twigs. The residues of some materials were more toxic than others to certain codling moth stages; for example, Stauffer R-3413 caused no egg mortality, but it was toxic to the other stages of codling moth; the toxicity of the residues of carbaryl to adults decreased more rapidly than its toxicity to eggs or larvae; and the residue of General Chemical GS-3707 was highly toxic to adults in comparison with its toxicity to eggs and larvae.

Guthion was more toxic over a longer period of time than any other material assayed, and even when it was applied at one-half the rate per acre usually recommended in spray bulletins, it was nearly so. To save table space, only the mortalities it caused during 6 weeks after application are given, although at 3 pounds per acre, it was still highly effective 7 and 8 weeks after application. Carbaryl also performed well but was not the equal of Guthion. Among the more promising newer materials, all stages considered, were Bayer compounds 37344, 47940, and 29493.

Insecticide residues remained on apple leaves, twigs, and fruit in toxic quantities over an uncommonly long period of time. This was largely because of lack of precipitation in central Washington. The average monthly precipitation at Yakima, Wash., from June to September was 0.37 inch.

CHEMICAL NAMES OF THE

PROPRIETARY MATERIALS TESTED

Bayer 22408 --------------	O,O-diethyl O-naphthalimido phosphorothioate
Bayer 29493 --------------	O,O-dimethyl O-[4-(methylthio)-m-tolyl] phosphorothioate
Bayer 34098 --------------	O-[4-(methylthio)-m-tolyl] dimethyl-phosphinothioate
Bayer 37344 --------------	4-(methylthio)-3,5-xylyl methylcarbamate
Bayer 39007 --------------	o-isopropoxyphenyl methylcarbamate
Bayer 41831 --------------	O,O-dimethyl O-4-nitro-m-tolyl phosphorothioate
Bayer 44646 --------------	4-dimethylamino-m-tolyl methylcarbamate
Bayer 47940 --------------	O-(3-chloro-4-cyanophenyl) O,O-dimethyl phosphorothioate
Eradex -------------------	2,3-quinoxalinedithiol cyclic trithiocarbonate
General Chemical GS-3707 ----------------	dimethyl 3-hydroxyglutaconate, dimethyl phosphate
Guthion ------------------	O,O-dimethyl S-(4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl) phosphorodithioate
Shell SD-5533 ------------	p-nitrobenzyl 3-hydroxycrotonate, dimethyl phosphate
Stauffer R-3413 ----------	S-(4,6-dimethyl-2-pyrimidinyl) O,O-diethyl phosphorodithioate

Table 1.--Mortality of codling moth stages determined by bioassay of the toxicity of insecticide residues on leaves, twigs, and immature fruits taken from orchard test plots at weekly intervals

Insecticides and quantities of active material applied per acre	Average percent mortality of codling moth stages caused by insecticide residues				
	Weeks after application of insecticide				
	2	3	4	5	6
Guthion, 3 lb.					
On leaves					
Adults	100.0	100.0	100.0	98.6	100.0
Eggs	89.3	92.5	96.3	78.3	78.5
Larvae	100.0	100.0	100.0	100.0	100.0
On apples, larvae	100.0	100.0	100.0	100.0	100.0
Guthion, 1.5 lb.					
On leaves					
Adults	--	95.8	96.8	86.1	61.4
Eggs	--	75.2	76.9	51.1	52.0
Larvae	--	100.0	94.4	68.9	86.7
On apples, larvae	--	100.0	100.0	100.0	99.4
Bayer 39197, 3 lb.					
On leaves					
Adults	--	91.5	89.6	95.7	86.4
Eggs	--	99.1	60.3	60.4	76.0
Larvae	--	97.8	93.2	98.4	84.3
On apples, larvae	--	97.8	100.0	96.6	98.8
Carbaryl, 6 lb.					
On leaves					
Adults	85.5	85.5	26.1	65.2	29.3
Eggs	55.1	41.2	21.0	13.3	20.6
Larvae	100.0	89.5	65.2	90.2	67.7
On apples, larvae	99.1	100.0	100.0	100.0	98.8
Bayer 37344, 3 lb.					
On leaves					
Adults	--	93.8	88.0	91.8	60.9
Eggs	--	56.9	35.7	46.2	49.9
Larvae	--	80.5	74.4	42.8	42.1
On apples, larvae	--	100.0	100.0	98.6	94.5

Table 1.--Continued.

Insecticides and quantities of active material applied per acre	Average percent mortality of codling moth stages caused by insecticide residues				
	Weeks after application of insecticide				
	2	3	4	5	6
General Chemical GS-3707, 4 lb.					
On leaves					
Adults	--	100.0	100.0	100.0	100.0
Eggs	--	26.5	34.1	19.3	13.1
Larvae	--	88.4	76.0	37.2	77.0
On apples, larvae	--	47.8	91.6	51.4	40.4
Bayer 41831, 3.2 lb.					
On leaves					
Adults	--	100.0	96.0	--	--
Eggs	--	21.5	47.8	--	--
Larvae	--	91.6	90.9	--	--
On apples, larvae	--	60.6	49.9	--	--
Bayer 47940, 3 lb.					
On leaves					
Adults	--	87.5	95.6	95.7	95.9
Eggs	--	20.1	2.3	9.7	4.6
Larvae	--	79.5	64.1	52.6	48.1
On apples, larvae	--	97.0	94.6	81.4	74.0
Bayer 29493, 3 lb.					
On leaves					
Adults	69.6	89.9	34.8	60.9	56.5
Eggs	42.7	31.1	14.6	7.8	7.6
Larvae	94.8	83.2	39.4	58.4	43.8
On apples, larvae	98.6	98.2	100.0	95.7	93.9
DDT, 8 lb. and parathion, 2 lb.					
On leaves					
Adults	--	81.3	86.7	95.7	83.0
Eggs	--	15.9	12.1	17.2	13.5
Larvae	--	39.5	52.1	40.2	15.1
On apples, larvae	--	91.7	77.6	76.1	69.7
Bayer 22408, 3 lb.					
On leaves					
Adults	43.9	59.5	39.1	34.8	18.5
Eggs	6.2	12.2	7.5	2.9	5.4
Larvae	75.5	77.0	48.8	77.2	65.7
On apples, larvae	79.2	85.8	84.1	88.5	87.5

Table 1.--Continued.

Insecticides and quantities of active material applied per acre	Average percent mortality of codling moth stages caused by insecticide residues				
	Weeks after application of insecticide				
	2	3	4	5	6
Phosphamidon, 6 lb.					
On leaves					
Adults	--	93.8	90.0	79.6	82.6
Eggs	--	15.9	23.1	33.8	12.2
Larvae	--	60.3	78.4	46.2	46.9
On apples, larvae	--	98.6	97.3	74.3	31.8
Stauffer R-3413, 3 lb.					
On leaves					
Adults	--	93.6	95.9	73.9	43.2
Eggs	--	0.1	0.0	0.0	0.0
Larvae	--	59.8	31.4	41.1	49.8
On apples, larvae	--	96.9	74.8	66.8	57.2
Bayer 39007, 3 lb.					
On leaves					
Adults	--	83.3	95.0	57.1	--
Eggs	--	20.7	31.8	12.9	--
Larvae	--	65.9	60.5	46.2	--
On apples, larvae	--	75.6	27.9	55.6	--
Bayer 30686, 4 lb.					
On leaves					
Adults	--	--	85.0	30.6	--
Eggs	--	--	26.8	18.6	--
Larvae	--	--	74.3	17.8	--
On apples, larvae	--	--	74.1	62.2	--
Bayer 44646, 3 lb.					
On leaves					
Adults	--	--	75.0	18.4	--
Eggs	--	--	23.5	15.4	--
Larvae	--	--	76.5	41.2	--
On apples, larvae	--	--	84.2	76.3	--
Endosulfan, 4 lb.					
On leaves					
Adults	--	82.6	96.0	--	--
Eggs	--	4.5	7.9	--	--
Larvae	--	69.2	46.7	--	--
On apples, larvae	--	38.5	20.7	--	--

Table 1.--Continued.

Insecticides and quantities of active material applied per acre	Average percent mortality of codling moth stages caused by insecticide residues				
	Weeks after application of insecticide				
	2	3	4	5	6
Phosphamidon, 2.4 lb.					
On leaves					
Adults	72.1	91.3	47.8	--	--
Eggs	24.0	70.8	21.7	--	--
Larvae	81.4	48.8	44.6	--	--
On apples, larvae	83.1	81.6	44.8	--	--
Diazinon, 4 lb.					
On leaves					
Adults	75.5	84.4	21.7	--	--
Eggs	21.9	6.9	3.0	--	--
Larvae	58.2	71.5	27.6	--	--
On apples, larvae	94.4	88.9	79.3	--	--
Ethion, 4 lb.					
On leaves					
Adults	64.6	30.4	0.0	--	--
Eggs	41.3	35.0	17.0	--	--
Larvae	60.4	67.2	27.6	--	--
On apples, larvae	72.5	72.8	54.2	--	--
Bayer 34098, 3 lb.					
On leaves					
Adults	71.2	59.0	30.4	--	--
Eggs	10.4	38.0	0.0	--	--
Larvae	69.9	49.4	16.8	--	--
On apples, larvae	68.0	59.9	8.6	--	--
Volck Supreme Oil, 2 gal. and ethion, 24 lb.					
On leaves					
Adults	8.7	0.3	--	--	--
Eggs	4.8	2.8	--	--	--
Larvae	36.9	24.8	--	--	--
On apples, larvae	60.3	50.5	--	--	--
Shell SD 5533, 2 lb.					
On leaves					
Adults	21.7	0.0	--	--	--
Eggs	2.6	11.1	--	--	--
Larvae	47.8	40.9	--	--	--
On apples, larvae	--	--	--	--	--

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